Available online at www.sciencedirect.com

ScienceDirect

Energy Procedia 100 (2016) 160 – 165

Energy Procedia

3rd International Conference on Power and Energy Systems Engineering, CPESE 2016, 8-12 September 2016, Kitakyushu, Japan

# Enzymatic saccharification of rice straw under influence of recycled ionic liquid pretreatments

Kraipat Cheenkachorn[1], Tom Douzou[2], Supacharee Roddecha[3], Prapakorn Tantayotai[4] and Malinee Sriariyanun[5,*]

[1]*Department of Chemical Engineering, Faculty of Engineering, KMUTNB, Thailand*
[2]*Department of Chemical Engineering, National Polytechnic Institute of Chemical Engineering and Technology, France*
[3]*Department of Chemical Engineering, Faculty of Engineering, Kasetsart University, Thailand*
[4]*Department of Biology, Faculty of Science, Srinakharinwirot, University, Thailand*
[5]*Department of Mechanical and Process Engineering, TGGS, KMUTNB, Thailand*

**Abstract**

Lignocellulosic biomass is one of a promising challenge for biorefinery processing with competitive costs to produce value added products, including biofuels and chemicals. We reported here that efficient ionic liquid (IL), 1-ethyl-3-methylimidazolium acetate ([C2mim][OAc]), pretreatments of rice straw can be achieved with 71.83 min of duration time, 128.4 °C of reaction temperature, and 5% w/w loading mass ratio. Enzymatic saccharification of IL- pretreated rice straw is significantly increased when compared to untreated ones, which yield glucose product about 90% yield. Additionally, we also report that using different types of anti-solvents, including methanol, water, and acetone, has different capability of cellulose recovery in regeneration step. The recyclability of [C2mim][OAc] using methanol as an anti-solvent is shown to retain more than 90% efficiency for 5 times without any modification in pretreatment process.



Peer-review under responsibility of the organizing committee of CPESE 2016

*Keywords:* Ionic liquid, Recycle, Pretreatment, Linocellulosic biomass

* Corresponding author. Tel.: +66 81 398 0439
*E-mail address:* macintous@gmail.com

1876-6102 
Peer-review under responsibility of the organizing committee of CPESE 2016
doi:10.1016/j.egypro.2016.10.159

## 1. Introduction

Lignocellulosic-based biomass, including agricultural wastes, has been produced in correlation with agricultural activities. After harvesting season, most of these residues were left on the field and burned out. In 2009, more than 55.61 million tons of agricultural wastes were left unused in Thailand [1]. These lignocellulosic-based biomass was estimated to yield potential energy capacity up to 732,534 TJ [1]. Based on this situation, it is important to develop the process to utilize these wastes by converting them to value-added products.

Lignocellulosic biomass mainly consists of celluloses, hemicelluloses, and lignin [2]. Cellulose and hemicellulose are biopolymers that could be hydrolyzed by lignocellulolytic enzymes to release sugars [3]. The sugar then could be used as raw materials to produce biofuels via biochemical processes and fermentations. One of the main bottle-neck step of the whole process is the hydrolysis step to produce sugars from lignocellulosic biomass because the physical and the chemical properties are barriers of hydrolytic enzymes [4]. Ionic liquid (IL) pretreatment is one of potential method due to its high efficiency to solubilizes cellulose, and its recyclability [5, 6]. However, one of the major issue for IL application in pretreatment is the cost of IL [7, 8]. Therefore, we aimed to study the effect of recycled IL, 1-ethyl-3-methylimidazolium acetate ([EMIM]Ac), on enzymatic saccharification of rice straw. The efficiency of the pretreatment condition was evaluated by analysis of reducing sugars released from hydrolyzed rice straw. The results of this work will be applicable for further experiments to find the optimum operational conditions to enhance the conversion of lignocellulosic biomass to fermentable sugars.

## 2. **Materials and methods**

### *2.1 Materials and chemicals*

Rice straw collected from a local field in Ayuthdhaya province, Thailand. Cellulase from *Trichoderma reesei* (Celluclast® 1.5L) and [EMIM]Ac were purchased from Sigma-Aldrich. Cellobiase from *Aspergillus niger* was purchased from Megazyme. Other chemicals used in this study were purchased from Ajax.

### *2.2 Optimization of [EMIM]Ac pretreatment and IL recycle*

Optimum condition of IL pretreatment was carried out with Response Surface Methodology (RSM). The main response factor was the concentration of reducing sugars yielded from hydrolysis step ($Y$). The independent variables were loading ratio of rice straw to [EMIM]Ac ($X_1$: 5–15% (w/w)), temperature ($X_2$:100 – 140 °C), and pretreatment time ($X_3$:30–90 min). A Box-Behnken design [9, 10] was applied here for the optimization to set for each variable with three test levels (max = +1, mid = 0, min = -1)(Table 1). Experimental data were analyzed using the statistical software, Design-Expert software version 7.0.0 (STAT-EASE Inc., Minneapolis, MN, USA), to fit the second-order polynomial regression model

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii} X_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij} X_i X_j \qquad (1)$$

where Y is the response variable (reducing sugars content), $\beta_0$ is the constant, $\beta_i$ is the linear coefficient, $\beta_i$ is the quadratic coefficient, and $\beta_{ij}$ is the two factor interaction coefficient.

Table 1. Value of independent variables with corresponded coded levels

| Factor | $X_1$ | $X_2$ | $X_3$ |
|---|---|---|---|
| Code | -1 | 0 | 1 |
| $X_1$: loading ratio of rice straw to [EMIM]Ac (%wt) | 100 | 120 | 140 |
| $X_2$: pretreatment temperature (ºC) | 30 | 45 | 60 |
| $X_3$: pretreatment time (min) | 5 | 10 | 15 |

For each reaction, 100 mg of the rice straw was mixed with IL, and then heated to set temperature with controlled time (Table 2). After heating, equal volume of distilled water was added as an anti-solvent. This sample was centrifuged to separate solid and liquid phases. Then washed sample was centrifuged again and this washing was repeated for three times. The solid-phase sample was dried in hot air oven. Each samples was enzymatic hydrolyzed using cellulase enzyme mixtures (containing 50 mM sodium citrate buffer (pH 4.7) with 4 ml of working volume, 0.1 g of pretreated biomass and 40 µl of 2 M sodium azide) [11, 12]. The reactions were incubated at 45 °C for 72 h in a 200 rpm shaking incubator. After digestion, the sample was centrifuged to separate the biomass from the liquid fraction. The liquid fraction was analyzed for reducing sugars by dinitrosalicylic (DNS) method [13].

To observe the recyclability of [EMIM]Ac in pretreatment, the optimum condition of pretreatment obtained from calculation of RSM analysis was performed to pretreat rice straw. After finishing first round of pretreatment, the used [EMIM]Ac was separated by centrifugation and recovered by adding three types of anti-solvents, including deionized water, acetone and methanol. The mixture of used [EMIM]Ac and anti-solvent was evaporated to remove anti-solvent and retrieve [EMIM]Ac for next round of pretreatment. The evaporation temperatures of anti-solvent were set at 100 °C, 56 °C and 65 °C for deionized water, acetone and methanol, respectively.

## 3. Results and discussion

### *3.1 Optimization of IL pretreatment*

To determine optimum working condition of rice straw pretreatment by [EMIM]Ac, the RSM with Box-Behnken design was applied (Table 2). First, experimental data was analyzed to select fit model. Then, the models were proceeded to ANOVA analysis (Table 3) to evaluate empirical relationship between the response and independent variables. Based on the p-value, the generated model was significant (p-value = 0.0026) to represent our experimental data with high $R^2$ value (0.9032). The mathematic model representing the pretreatment parameter to sugar concentration and optimum working condition was calculated and represented as a contour plot (Table 4 and Fig. 1). Based on this model, the maximum % recovered sugar was obtained when using pretreatment condition at 5% loading ratio, 128.4 °C temperature, 71.83 min Time. This optimum working condition was carried out for experiment of [EMIM]Ac recycling.

Table 2. Experimental design to test the effects of pretreatment parameters (loading ratio ($X_1$, %wt), temperature ($X_2$, °C) and time ($X_3$, min)) on reducing sugar concentration (Y, mg/ml) using [EMIM]Ac.

| Run | $X_1$ | $X_2$ | $X_3$ | Y |
|---|---|---|---|---|
| 1 | 5 | 100 | 60 | 5.651 |
| 2 | 15 | 100 | 60 | 4.158 |
| 3 | 5 | 140 | 60 | 13.376 |
| 4 | 15 | 140 | 60 | 7.333 |
| 5 | 5 | 120 | 30 | 7.951 |
| 6 | 15 | 120 | 30 | 5.474 |
| 7 | 5 | 120 | 90 | 10.817 |
| 8 | 15 | 120 | 90 | 11.051 |
| 9 | 10 | 100 | 30 | 3.756 |
| 10 | 10 | 140 | 30 | 9.963 |
| 11 | 10 | 100 | 90 | 6.849 |
| 12 | 10 | 140 | 90 | 7.809 |
| 13 | 10 | 120 | 60 | 11.826 |
| 14 | 10 | 120 | 60 | 10.653 |
| 15 | 10 | 120 | 60 | 11.400 |
| 16 | 10 | 120 | 60 | 10.795 |
| 17 | 10 | 120 | 60 | 11.186 |

Table 3. Analysis of variance (ANOVA) for the adjusted quadratic model of sugar concentration released from pretreated rice straw.

| [EMIM]Ac pretreatment | | | | | |
|---|---|---|---|---|---|
| Source | Sum of Squares | df | Mean Square | F Value | p-value |
| Model | 1760.98 | 5 | 352.20 | 7.60 | 0.0026 |
| $X_1$ | 199.50 | 1 | 199.50 | 4.31 | 0.0622 |
| $X_2$ | 680.81 | 1 | 680.81 | 14.70 | 0.0028 |
| $X_3$ | 183.58 | 1 | 183.58 | 3.96 | 0.0719 |
| $X_2^2$ | 507.29 | 1 | 507.29 | 10.95 | 0.0070 |
| $X_3^2$ | 156.36 | 1 | 156.36 | 3.38 | 0.0933 |
| Residual | 509.57 | 11 | 46.32 | - | - |

Table 4. Mathematical models represent the influence of pretreatment parameters on the reducing sugar concentration and optimal condition of [EMIM]Ac pretreatment.

| | |
|---|---|
| [EMIM]Ac pretreatment | Mathematical models: Sugar concentration (mg/ml) = -429.80831 - 0.99874xConc + 7.03793xTemp + 0.97107xTime - 0.027403xTemp$^2$ - 6.76158x10$^{-3}$xTime$^2$ |
| | Optimal pretreatment condition: 5% loading ratio, 128.4 ºC temperature, 71.83 min time |

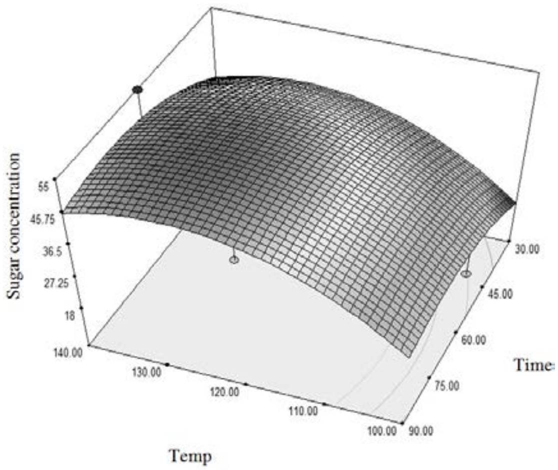


Fig. 1. Response surface plots of [EMIM]Ac pretreatment represent effects of the interaction between the independent variables on the sugar yields.

### *3.2 IL recyclability with different anti-solvents*

In this study, three types of anti-solvents, including deionized water, acetone and methanol, were selected to recover [EMIM]Ac from pretreated rice straw mixtures. To observe the efficiency of recycled [EMIM]Ac, optimum working condition of pretreatment was performed (Table 4). After solid biomass was removed, each anti-solvents was added into the mixture with 1:1 ratio separately. The recovered [EMIM]Ac was evaporated to remove anti-solvent, and reused for pretreatment of rice straw for the next round. Each pretreated biomass from each round of recycle was hydrolyzed and sugar products were measured by DNS method (Fig. 2). From the results of reducting sugar contents obtained from hydrolysis reactions, methanol was the best anti-solvent because the highest amounts of sugars were obtained, except the 5th and 6th cycle. However, the efficiencies of 5th and 6th cycle were deviated from average of efficiency for 7.74%. For deionized water set, the efficiency of recovery was quite constant from 1st cycle to 6 cycles. Among all three types of anti-solvents here, acetone showed the lowest potential for [EMIM]Ac recovery because at the 6th cycle, the sugar yield dropped from 5th cycle for 21.17%, which was the most reduction observed in three solvents.

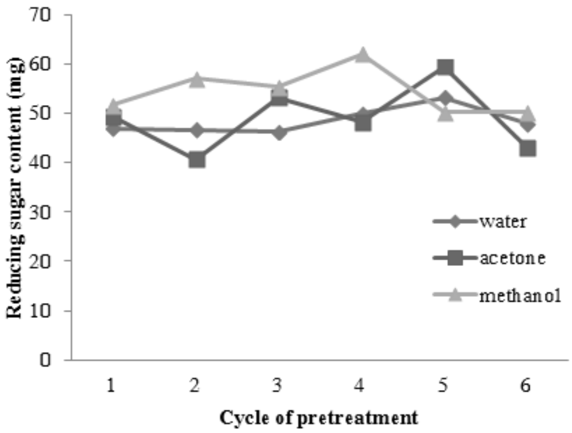


Fig. 2. Reducing sugar contents obtained from hydrolyzed rice straws that were pretreated with recycled [EMIM]Ac by using three different types of anti-solvents.

## 4. Conclusion

In this study, the effect of [EMIM]Ac pretreatment to enhance saccharification of rice straw was investigated through RSM analysis. Based on the goal to apply the use of IL pretreatment to biorefinery process, the optimization experiment is necessary to minimize the use of high cost IL, but still achieve high sugar yields from hydrolysis step. Additionally, we also investigated different types of anti-solvents to recover used IL to make it reusable for pretreatment leading to reduction of the IL cost. However, further study should be done to calculate the cost of recycling process whether it is feasible to biorefinery application

## Acknowledgements

The authors would like to thank King Mongkut's University of Technology North Bangkok (Research Grant contract No. KMUTNB-GOV-58-22, KMUTNB-NRU-59-08) and Srinakharinwirot University (Research Grant contract No.307/2559) for financial support of this work.

## References

[1] http://www.dede.go.th (achieved on 10 Jan 2016).
[2] Nigam P, Gupta N, Anthwal A: Pre-treatment of agroindustrial residues. In: Biotechnology for agro-industrial residues utilization. Netherlands: Springer; 2009: 13-33.
[3] Lynd L, Weimer PJ, van Zyl WH, Pretorius IS: Microbial cellulose utilization: Fundamentals and biotechnology. Microbiol Molec Biol Rev 2002; 66(3):506-577.
[4] Agbor V, Cicek N, Sparling R, Berlin A, Levin D: Biomass pretreatment: Fundamentals toward application. Biotechnol Adv 2011; 29:675-685.
[5] Wang Y, Song H, Peng L, Zhang Q, Yao S. Recent developments in the catalytic conversion of cellulose. Biotechnol Biotechnol Equip; 2014, 28(6):981-988.
[6] Sriariyanun M, Tantayotai P, Yasurin P, Pornwongthong P, Cheenkachorn K. Production, purification and characterization of an ionic liquid tolerant cellulase from Bacillus sp isolated from rice paddy field soil. Electron J Biotechn.2016; 19:23-28.
[7] Engel P, Mladenov R, Wulfhorst H, Jager G, Spiess AC. Point by point analysis: how ionic liquid affects the enzymatic hydrolysis of native and modified cellulose. Green Chem.2010; 12:1959–1966.

[8] Ang T, Ngoh GC, Chua AS, Lee MG. Elucidation of the effect of ionic liquid pretreatment on rice husk via structural analyses. Biotechnol Biofuels.2012; 5(1):67.
[9] Bringans S, Eriksen S, Kendrick T, Gopalakrishnakone P, Livk A, Lock R, R. L. Proteomic analyses of the venom of Heterometrus longimanus (Asian black scorpion) Proteomics.2008; 8:1081-1096.
[10] Sriariyanun M: Response surface methodology for optimization of biodiesel production by Acinetobacter baylyi. KMUNB Int J Appl Sci Technol; 2014, 7(4): p. 47-52.
[11] Sriariyanun M, Yan Q, Nowik I, Cheenkachorn K, Phusantisampan T, Modigell M. Efficient pretreatment of rice straw by combination of screw-press and ionic liquid to enhance enzymatic hydrolysis Kasetsart J 2015; 49:146-154.
[12] Amnuaycheewa P, Hengaroonprasan R, Rattanaporn K, Kirdponpattara S, Cheenkachorn K, Sriariyanun M. Enhancing enzymatic hydrolysis and biogas production from ricestraw by pretreatment with organic acids. Indus Crops Prod. 2016; 87:247-254.
[13] Miller G. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem.1959; 31(3):426-428.